CH2MHILL

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29 July 2002

Mr. Carl Goldstein
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CMD-1
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American Samoa Government
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Enclosed is the required report for the bioassay test results for the March 2002 effluent sampling for Joint Cannery Outfall in American Samoa. The sampling and analysis were carried out without problems. The result are similar to the past bioassay test results.

Please call us if you have any questions or comments on the enclosed report,

Sincerely,

Karen A. Glatzel

Cc: Jim Cox, COS International; Herman Gebauer, COS; Brett Ransby, COS; John Brown, Heinz; Phil Thirkel, StarKist Samoa; Joe Carney, StarKist Samoa; David Wilson, CH2M HILL.

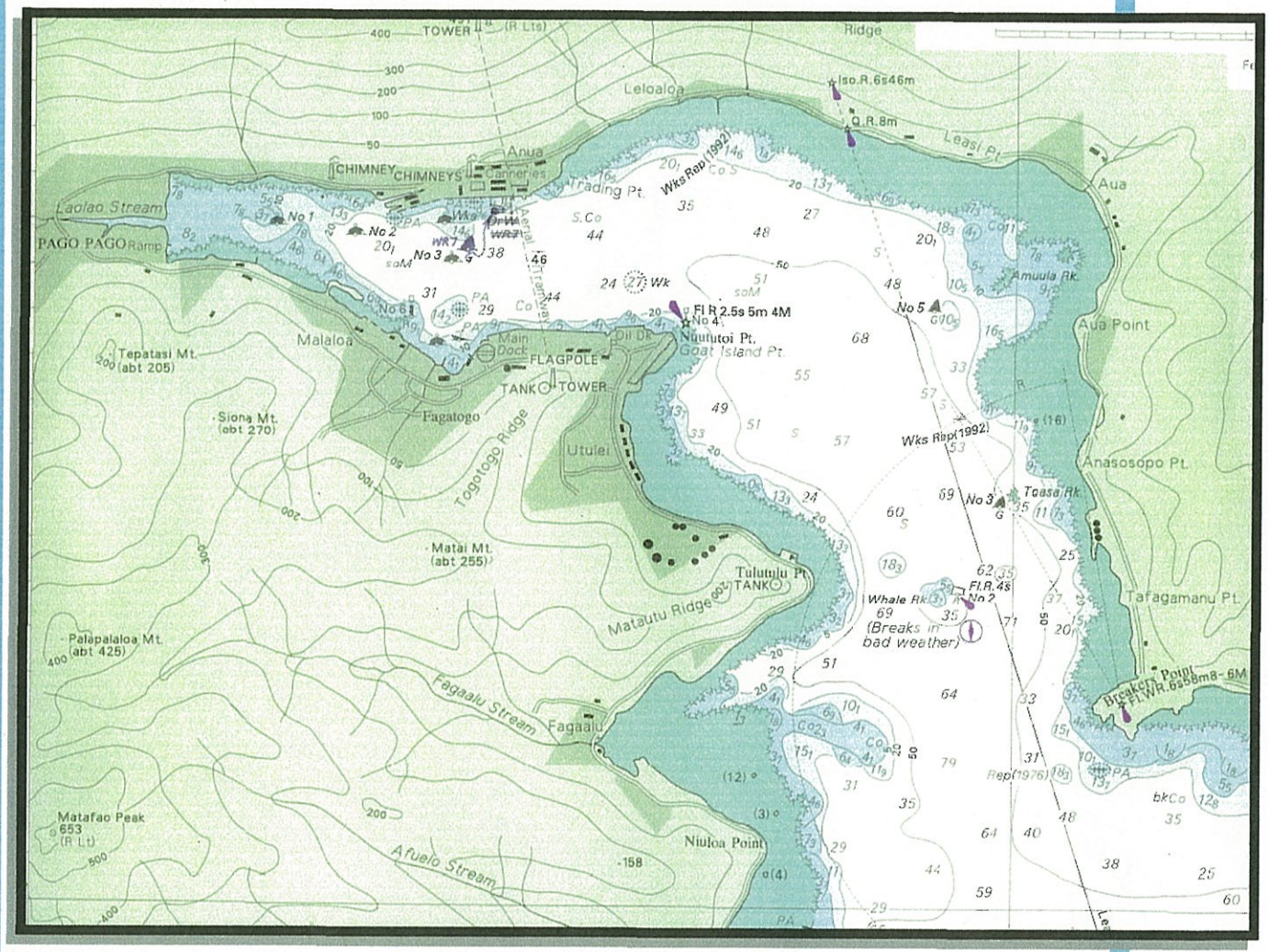
Encl: Effluent Bioassay Results for October 2001 Sampling

SAMOA JOINT CANNERY OUTFALL

2002 Non-tradewind Season

EFFLUENT BIOASSAY TEST RESULTS

March 2002 Sampling



29 July 2002

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TECHNICAL MEMORANDUM

BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT MARCH 2002 SAMPLING

Prepared For: StarKist Samoa (NPDES Permit AS0000019)
COS Samoa Packing (NPDES Permit AS0000027)

Prepared By: Steve Costa
Karen Glatzel

Date: 29 July 2002

Distribution: Carl Goldstein
United States Environmental Protection Agency, Region 9
Peter Peshut
American Samoa Environmental Protection Agency

Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in March 2002. The testing is required by the NPDES Permits that became effective in January 2001. This is the third required semiannual test required by the current permits and the seventeenth semiannual test conducted since testing for the Joint Cannery Outfall began in 1993.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES Permits requires that semiannual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the combined joint cannery effluent discharge into Pago Pago Harbor.

Study Approach

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests conducted in the past. All comments from U.S. EPA have been incorporated into the sampling and sample handling standard operating procedures (SOP) or have been incorporated into the procedures used by the laboratory doing the test. The comments, responses, and SOP have been documented in previous reports.

The permit conditions require that the bioassay tests be conducted with the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests, the permit specifies the substitute species, *Mysidopsis bahia,* which now has been renamed *Americamysis bahia.* For the March 2002 sampling, *Penaeus vannami* was not available and *Americamysis bahia* was used.

Effluent samples were collected from the StarKist Samoa and COS Samoa Packing facilities as 24-hour composite samples. The acute effluent bioassay test was conducted using a combined, flow-weighted, composite effluent sample made up from the effluent samples from both canneries, as allowed by the permit condition. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 0900 on 14 March 2002 and 0600 on 15 March 2002, 24-hour flow-weighted composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites. Detailed sampling procedures are described in the established SOP for cannery effluent sampling.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24-hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times, effluent flow rates, and the relative effluent flow volumes calculated from plant flow records are summarized in Table 1. The relative effluent flow volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL to the testing laboratory. The chain-of-custody form is provided in Attachment I.

Table 1
StarKist Samoa and COS Samoa Packing
24-hour Composite Effluent Sample for Bioassay Testing
March 2002

Grab Sample Number	COS Samoa Packing		StarKist Samoa		COS Samoa Packing Percent of Total Flow	StarKist Samoa Percent Of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
1	14 Mar 2002 0900	0.68	14 Mar 2002 0900	1.89	2.7	7.5
2	1200	1.20	1200	1.18	4.8	4.7
3	1500	0.96	1500	2.10	3.8	8.4
4	1800	0.96	1800	2.16	3.8	8.6
5	2100	0.64	2100	2.73	2.6	10.9
6	15 Mar 2002 0000	0.64	15 Mar 2002 0000	3.48	2.6	13.9
7	0300	0.64	0300	2.96	2.6	11.8
8	0600	0.68	0600	2.17	2.7	8.6
Total		6.40		18.67	25.6	74.4
Mean		0.80		2.33		

Bioassay Testing Procedures

EnviroSystems, Inc. located in Hampton, New Hampshire conducted the bioassay tests. The testing procedures and results of the bioassay tests are provided in the Laboratory report included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA documents Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA/600/4-90/027F), August 1993 as the sources of methods for conducting the test. The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports.

The test organisms were ≤ 5 days old and the test temperature was to be held at 20 ±1°C , but actual temperatures ranged from 17°C to 22°C. Salinity was adjusted to

24 ppt at the start of the test and ranged between 25 and 30 ppt. Demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD) had been discussed and documented in previous bioassay technical memoranda which describe the first two tests conducted in 1993. Therefore, all of the bioassay test chambers were continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). The test was renewed with oxygenated sample at 48 hours.

Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, and temperature. Total residual chlorine and ammonia were measured. Reference toxicant tests using sodium dodecyl sulfonate (SDS) are conducted regularly by ESI with the last one completed on February 20, 2002 and results were within one standard deviation of the historic laboratory mean.

Summary Results: Americamysis bahia Effluent Bioassay

All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the 96-hour LC_{50} for the effluent tested was 16.1 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 12.5 percent and the Least Observable Effects Concentration (LOEC) was 25 percent. Results on a daily basis are summarized in Table 2.

Table 2
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results March 2002 Sampling

Exposure Time	Parameter		
	LC_{50}	NOEC	LOEC
24 hours	21.7%	12.5%	25%
48 hours	21.7%	12.5%	25%
72 hours	21.7%	12.5%	25%
96 hours	16.1%	12.5%	25%

Discussion

Table 3 summarizes the results of the effluent bioassay tests for the samples collected in the March 2002 sampling compared to the previous bioassay tests. The

LC_{50}, NOEC and LOEC are within the range obtained from previous reports where *Mysidopsis bahia* was used in place of *Penaeus vannami*.

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for March 2002 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 16.1%, which was observed in March 2002, corresponds to a dilution of 6.25:1, which is achieved within a second and within 1-meter of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Table 3
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results

Date	Species	Parameters		
		LC 50	NOEC	LOEC
2/93	*Penaeus vannami*	4.8%[1]	3.1%	6.25%
10/93	*Penaeus vannami*	15.67%	3.1%	6.25%
2/94	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94	*Mysidopsis bahia*[2]	31.2%	25%	50%
3/95	*Penaeus vannami*	14.8%	6.25%	12.5%
3/95	*Mysidopsis bahia*[3]	10.8%	6.25%	12.5%
2/96	*Penaeus vannami*	>50%	>50%	>50%
2/96	*Mysidopsis bahia*[3]	28.36%	12.5%	25%
3/96	*Penaeus vannami*	44.4%	25%	50%
11/96	*Penaeus vannami*	7.11%	3.1%	6.25%
03/97	*Penaeus vannami*	39.36%	12.5%	25%
09/97	*Penaeus vannami*[4]	12.3%	6.25%	12.5%
06/98	*Mysidopsis bahia*[2]	17.2%	6.25%	12.5%
11/98	*Mysidopsis bahia*[2]	15%	6.25%	12.5%
02/00	*Mysidopsis bahia*[2]	20%	6.25%	12.5%
08/00	*Mysidopsis bahia*[2]	17.1%	3.1%	6.25%
03/01	*Americamysis bahia*[5]	13.8%	12.5%	25%
10/01	*Americamysis bahia*[6]	37.5%	25.0%	50.0%
3/01	***Americamysis bahia***[6]	**16.1%**	**12.5%**	**25%**

[1]The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

[2]*Mysidopsis bahia* used as substitutes because *Penaeus vannami* not available: as directed and approved by U. S. EPA.

[3]*Mysidopsis bahia* used in addition to *Penaeus vannami* as described in text of technical memorandums reporting test results. Only one species is required by the permit conditions.

[4]Stage 1 (3 mm) *Penaeus vannami* were used for testing because older Stage 7 and 8 (8-10 mm) *Penaeus vannami* were not available.

[5]*Mysidopsis bahia* renamed *Americamysis bahia.* Results indicate increased toxicity because of low DO in renewal concentrations as renewal water was not aerated prior to use

[6] *Mysidopsis bahia* renamed *Americamysis bahia*

ATTACHMENT I

Chain-of-Custody

CH2M HILL
APPLIED SCIENCES LABORATORY

CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES

CH2M Hill Project #: 147323.JC.02.NT
Purchase Order #:

Project Name: JOINT CANNERY OUTFALL SAMOA

Company Name/CH2M HILL Office:

Project Manager & Phone #: Mr. [ ] Ms. [ ] Dr. [ ] STEVE COSTA 707-677-6123
Report Copy to: SAME

Requested Completion Date:
Sampling Requirements: SDWA ☐ NPDES ☐ RCRA ☐ OTHER ____
Sample Disposal: Dispose ☐ Return ☐

LAB TEST CODES

SHADED AREA – FOR LAB USE ONLY
Lab 1 #: | Lab 2 #:
Quote #: | Kit Request #:
Project #:
No. of Samples: | Page of
Login: | LIMS Ver

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	Matrix AIR	CLIENT SAMPLE ID (9 CHARACTERS)	# OF CONTAINERS	ANALYSES REQUESTED: MYSID ACUTE (96 hr) BIOASSAY	REMARKS	LAB 1 ID	LAB 2 ID
3/14/02		X		X			JCO-02-NT	1	X	Dilutions: 5, 6, 25, 12, 6, 3 1/8		

Sampled By & Title (Please sign and print name): [signature]	Date/Time: 3/14-15/02	Relinquished By (Please sign and print name): Steve Costa	Date/Time: 3/15/02
Received By (Please sign and print name):	Date/Time:	Relinquished By (Please sign and print name):	Date/Time:
Received By (Please sign and print name):	Date/Time:	Relinquished By (Please sign and print name):	Date/Time:
Received By (Please sign and print name):	Date/Time:	Shipped Via: UPS BUS Fed-Ex Hand Other DHL	Shipping # 8190660203
Work Authorized By (Please sign and print name):	Remarks:		

QC Level: 1 2 3 Other: ____

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Ana Req	TEMP
Cust Seal	Ph

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Company name: [illegible] HILL
Shipper's name: JIM CRAWFORD
Address: 9TH FL, 777 108TH AVE NE, BELLEVUE WA | RAINMAKER HOTEL, PAGO PAGO, AMERICAN SAMOA 96799
Zip code (required):
Phone/Fax/Telex circle one: (684) 453-5000

2 To (Recipient)

Company name: ENVIROSYSTEMS INC
Attention: PETRA KARBE
Delivery address: ONE LAFAYETTE ROAD, HAMPTON, N.H.
Zip/Postcode (required): 03842
Phone/Fax/Telex circle one: 603-926-3345

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Services
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- [ ] INTERNATIONAL DOCUMENT
- [x] INTERNATIONAL NON DOCUMENT
- [ ] WORLDMAIL 1st / APM / 2nd *circle one*

Special Services *extra charges may apply*
- [ ] SATURDAY DELIVERY
- [ ] POD
- [ ] OTHER .... *specify*

Payment Options *not all options available to all countries*
- [x] Shipper's account
- [ ] Recipient
- [x] Third party

Acct. No. ....
- [ ] Cash / Check / Credit Card *circle one*

No. ....
Expires .... Type ....
Shipment insurance *if desired, enter amount below*
U.S. $ .... *Insured value*

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ATTACHMENT II

ESI Laboratory Report

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT MARCH 2002

American Samoa Joint Cannery Outfall

Prepared For

CH2M Hill, Incorporated
P.O. Box 1238
Trinidad, California 95570-1238

By

EnviroSystems, Incorporated
One Lafayette Road
Hampton, New Hampshire 03842

March 2002
Reference Number CH2M10213-02-03

STUDY NUMBER 10213

EXECUTIVE SUMMARY

The following summarizes the results of acute exposure bioassays performed from March 22-26, 2002 in support of the NPDES biomonitoring requirements of the American Samoa Joint Cannery Outfall. The 96 hour acute definitive assays were conducted using the marine species, *Americamysis bahia.*

Acute Toxicity Evaluation

Species	Exposure	LC-50	NOEC	LOEC
Americamysis bahia	24-Hours	21.7%	12.5%	25%
	48-Hours	21.7%	12.5%	25%
	72-Hours	21.7%	12.5%	25%
	96-Hours	16.1%	12.5%	25%

COMMENTS:

Results reflect test concentrations after salinity adjustment. See Section 2.3.

Authorized Signature: _____ 4/29/02

President ~ EnviroSystems, Incorporated Date

TOXICOLOGICAL EVALUATION
OF A TREATED EFFLUENT:
BIOMONITORING SUPPORT FOR A NPDES PERMIT
MARCH 2002

American Samoa Joint Cannery Outfall

1.0 INTRODUCTION

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test animals are exposed to each effluent concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration which kills half of the test animals. Samples with high LC-50 values are less likely to cause significant environmental impact. The acute no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) document the highest and lowest effluent concentrations that have no impact and a significant impact on the test species, respectively.

This report presents the results of acute toxicity tests conducted on an effluent sample collected from the American Samoa Joint Cannery Outfall. Testing was based on programs and protocols developed by the US EPA (1993) and involved conducting 96 hour acute static renewal toxicity tests with the marine species, *Americamysis bahia*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire.

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed by the EPA to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms, and for the analysis of water samples. See Section 4.0 for a list of references.

2.2 Test Species

A. bahia, ≤5 days old, were from cultures maintained by ESI. Test organisms were transferred to test chambers by large bore pipet, minimizing the amount of water added to test solutions.

2.3 Effluent and Dilution Water

The effluent sample used in the assay was identified as "JCO-02-NT." Sample collection information is provided in Table 4. Upon receipt, the sample was stored at 4°C. All sample material used in the assay was warmed to 20±1°C prior to preparing test solutions. Total residual chlorine (TRC) was measured using amperometric titration (MDL 0.05 mg/L). As the effluent sample contained <0.05 mg/L, TRC dechlorination with sodium thiosulfate was not required (EPA 1993). Aliquots of the undiluted effluent sample were collected for ammonia analysis when the sample arrived and again prior to renewal. At arrival, the effluent sample had a salinity of 13‰. Salinity of the effluent was increased to 24‰ by the addition of artificial sea salts. Test concentrations for the assays were 50%, 25%, 12.5%, 6.25% and 3.1% effluent with dilution water control.

The dilution water used in this assay was collected by ESI from its sea water system. The water is pumped from the Hampton Estuary on the flood tide, filtered through a high volume sand filter, and stored in 3000 gallon polyethylene tanks. The water is classified as Class A by the State of New Hampshire and has been used for culture of test organisms for over 20 years. Sea water used in the assay had a salinity of 24‰ and a TRC of <0.05 mg/L.

2.4 Acute Toxicity Tests

The 96 hour acute static renewal toxicity tests were conducted at 20±1°C with a photoperiod of 16:8 hours light:dark. Test chambers for the acute assays were 250 mL glass beakers containing 200 mL test solution in each of 5 replicates, with 10 organisms/replicate. Survival, dissolved oxygen, pH, salinity and temperature were measured daily in all replicates. Test solutions were renewed after 48 hours using effluent from the start sample. Mysid shrimp were fed <24 hour old brine shrimp on a daily basis.

2.5 Data Analysis

At 24 hour intervals, survival data was analyzed to assess toxicity using a program developed by Stephan (1982). LC-50 values were computed using the Spearman-Karber, Probit, Binomial, and Moving Average computation methods. If survival in the highest test concentration was >50%, LC-50 values were obtained by direct observation of the raw data. The NOEC was determined as the highest test concentration which caused no significant mortality while the LOEC was determined as the lowest concentration that did cause significant mortality.

2.6 Quality Control

As part of the laboratory quality control program, standard reference toxicant assays are conducted on a regular basis for each test species. These results provide relative health and response data while allowing for comparison with historic data sets. A forty-eight hour acute reference toxicant assay was performed with *A. bahia* on February 20, 2002. Results of this assay was within one standard deviation of its respective historic mean. See Table 2 for details.

3.0 RESULTS

Results of the acute exposure bioassay conducted using the mysid shrimp are summarized in Table 1. A summary of reference toxicant data for the test species is presented in Table 2. Effluent and dilution water characteristics are presented in Table 3. Sample collection information is provided in Table 4. Table 5 provides a summary of historic data associated with the discharge. Support data are included in Appendix A.

3.1 Acute Toxicity Test - *Americamysis bahia*

There was 100% survival in laboratory diluent control after 24 hours exposure and 98% survival after 48, 72, and 96 hours exposure. These results are an indication of healthy test organisms and that the dilution water had no adverse impact on the outcome of the assay.

Table 1 provides a summary of the acute exposure data and results.

3.2 Summary

The salinity adjusted effluent sample for the American Samoa Joint Cannery site exhibited signs of acute toxicity to the mysid shrimp, *Americamysis bahia,* during the 96 hour exposure period.

4.0 LITERATURE CITED

APHA. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. Washington D.C.

Stephan, C. 1982. Documentation for Computing LC-50 Values with a Mini Computer. Unpublished.

US EPA. 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms.* Fourth Edition. EPA/600/4-90/027F.

US EPA. 2001. *Attachment G: NPDES Whole Effluent Toxicity Testing, Monitoring and Reporting Tips and Common Pitfalls.* Dated December 2001. US EPA Region I Offices, Boston, Massachusetts.

TABLE 1. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2002.

Concentration % Effluent	Exposure	Replicates A	B	C	D	E	Mean	Standard Deviation	Coefficient of Variation
Lab Control	Start	10	10	10	10	10	100%	0.000	0.00%
	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	9	10	10	98%	0.447	4.56%
	72 Hours	10	10	9	10	10	98%	0.447	4.56%
	96-Hours	10	10	9	10	10	98%	0.447	4.56%
3.1%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	9	10	9	9	10	94%	0.548	5.83%
6.25%	24-Hours	10	7	8	9	8	84%	1.140	13.57%
	48-Hours	10	7	8	9	8	84%	1.140	13.57%
	72 Hours	10	7	8	9	8	88%	1.304	14.82%
	96-Hours	9	7	8	8	8	80%	0.707	8.84%
12.5%	24-Hours	10	10	0	9	9	76%	4.278	56.29%
	48-Hours	10	10	0	9	9	76%	4.278	56.29%
	72 Hours	10	10	0	9	9	76%	4.278	56.29%
	96-Hours	10	10	0	5	9	68%	4.324	63.59%
25%	24-Hours	10	8	0	0	0	36%	4.980	138.33%
	48-Hours	10	8	0	0	0	36%	4.980	138.33%
	72 Hours	10	8	0	0	0	36%	4.980	138.33%
	96-Hours	8	5	0	0	0	26%	3.715	142.88%
50%	24-Hours	0	0	0	0	0	0%	0.000	-
	48-Hours	0	0	0	0	0	0%	0.000	-
	72 Hours	0	0	0	0	0	0%	0.000	-
	96-Hours	0	0	0	0	0	0%	0.000	-

SUMMARY OF ENDPOINTS

Exposure Period	LC-50	NOEC	LOEC
24-Hours	21.71% (18.3-25.7)	12.5%	25.0%
48-Hours	21.71% (18.3-25.7)	12.5%	25.0%
72-Hours	21.71% (18.3-25.7)	12.5%	25.0%
96-Hours	16.13% (13.5-19.3)	12.5%	25.0%

TABLE 2. Summary of Reference Toxicant Data. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2002.

Concentrations Expressed as mg/L Sodium Dodecyl Sulfate

Species	Date	LC-50	Historic Mean	Number of Tests	±1 STD Deviation	±2 STD Deviation
A. bahia	02/20/02	16.5	19.8	134	4.35	8.70

TABLE 3. Summary of Effluent and Diluent Characteristics. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2002.

Parameter	Units	EFFLUENT	DILUENT
Salinity - on Arrival	‰	13	24
After Salinity Adjustment ‡	‰	24	-
pH - on Arrival	SU	6.71	8.00
After Salinity Adjustment ‡	SU	7.16	-
TRC	mg/L	<0.05	<0.05
Dissolved Oxygen	mg/L	1.2	7.6
Ammonia - at Start	mg/L as N	66.7	<0.10
Unionized Ammonia	mg/L as N	0.135	-
Ammonia - at Start- Salinity Adjusted ‡	mg/L as N	35.0	-
Unionized Ammonia ‡	mg/L as N	0.214	-
Ammonia - at 48 Hours ‡	mg/L as N	31.9	<0.10

TABLE 4. Summary of Sample Collection Information. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2002.

Sample Description	Type	Collection Date	Collection Time	Receipt Date	Receipt Time	Arrival Temp °C
EFFLUENT	Comp	03/14-15/02	ND	03/22/02	1325	15

COMMENTS:

‡ - Analysis performed on 50% effluent, the highest concentration tested.
ND - No data recorded on chain of custody.

TABLE 5. Summary of StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results. American Samoa Joint Cannery Outfall Effluent Evaluation. March 2002.

Date	Species	96-Hour Endpoints		
		LC-50	NOEC	LOEC
02/93[1]	*Penaeus vannami*	4.8%	3.1%	6.25%
10/93[1]	*Penaeus vannami*	15.67%	3.1%	6.25%
02/94[1]	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94[1]	*Americamysis bahia*	31.2%	25.0%	50.0%
03/95[1]	*Penaeus vannami*	14.8%	6.25%	12.5%
03/95[1]	*Americamysis bahia*	10.8%	6.25%	12.5%
02/96[1]	*Penaeus vannami*	>50.0%	>50.0%	>50.0%
03/96[1]	*Penaeus vannami*	44.4%	25.0%	50.0%
11/96[1]	*Penaeus vannami*	7.11%	3.1%	6.25%
03/97[1]	*Penaeus vannami*	39.36%	12.5%	25.0%
09/97[1]	*Penaeus vannami*	12.3%	6.25%	12.5%
06/98[1]	*Americamysis bahia*	17.2%	6.25%	12.5%
11/98[1]	*Americamysis bahia*	15.0%	6.25%	12.5%
02/00[1]	*Americamysis bahia*	20.0%	6.25%	12.5%
08/00[1]	*Americamysis bahia*	17.1%	3.1%	6.25%
03/01[2]	*Americamysis bahia*	13.81%	12.5%	25.0%
03/02[2]	*Americamysis bahia*	16.13%	12.5%	25.0%

Notes:

1. Assays conducted by Advanced Biological Testing, Inc., Rohnert Park, California

2. Assay conducted by EnviroSystems, Inc., Hampton, New Hampshire

APPENDIX A

DATA SHEETS

STATISTICAL SUPPORT

METHODS USED IN NPDES PERMIT BIOMONITORING TESTING

Parameter	Method
Acute Exposure Bioassays	
Ceriodaphnia dubia, Daphnia pulex	EPA 600/4-90/027
Pimephales promelas	EPA 600/4-90/027
Americamysis bahia	EPA 600/4-90/027
Menidia beryllina, Cyprinodon variegatus	EPA 600/4-90/027
Chronic Exposure Bioassays	
Ceriodaphnia dubia	EPA 600/4-91/002, 1002.0
Pimephales promelas	EPA 600/4-91/002, 1000.0
Cyprinodon variegatus	EPA 600/4-91/003, 1004.0
Menidia beryllina	EPA 600/4-91/003, 1006.0
Arbacia punctulata	EPA 600/4-91/003, 1008.0
Champia parvula	EPA 600/4-91/003, 1009.0
Trace Metals:	
ICP Metals	EPA 200.7/SW 6010
Hardness	Standard Methods 20th Edition - Method 2340 B
Wet Chemistries:	
Alkalinity	Standard Methods 20th Edition - Method 310.1
Chlorine, Residual	Standard Methods 20th Edition - Method 4500CLD
Total Organic Carbon	Standard Methods 20th Edition - Method 5310.6
Specific Conductance	Standard Methods 20th Edition - Method 2510B
Nitrogen - Ammonia	Standard Methods 20th Edition - Method 4500NH3G
pH	Standard Methods 20th Edition - Method 4500H+B
Solids, Total (TS)	Standard Methods 20th Edition - Method 2540.B
Solids, Total Suspended (TSS)	Standard Methods 20th Edition - Method 2540D
Dissolved Oxygen	Standard Methods 20th Edition - Method 4500-O G

ACUTE BIOASSAY DATA SUMMARY

STUDY: 10213	SAMPLE RECEIVED: 3/22/05	"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES						
CLIENT: CH2M Hill	TEST ORGANISM: *A. bahia*		TRC	AMM 0 HR✖	AMM 48 HR✖	pH	DO	Salinity
SAMPLE: American Samoa	ORGANISM SUPPLIER: ESI	EFFLUENT	<0.05	LH	LH	6.71	1.2	13
DILUENT: LAB SALT	ORGANISM BATCH/AGE: 1-5days	DILUENT	<0.05	LH	LH	8.00	7.6	34

SALINITY ADJUSTMENT RECORD (IF APPLICABLE): ML EFFLUENT + G SEA SALTS = 100% ACTUAL PERCENTAGE

CONC	REP	SURVIVAL					✦DISSOLVED OXYGEN (MG/L)✦						PH (SU)						TEMPERATURE (°C) (20°C)						SALINITY (ppt)					
		0	24	48	72	96	0	24	48◇	48☆	72	96	0	24	48◇	48☆	72	96	0	24	48◇	48☆	72	96	0	24	48◇	48☆	72	96
LAB	A	10	10	10	10	10	7.6	7.6	7.8	7.5	7.7	5.5	8.00	8.03	8.08	7.78	7.92	7.63	22	17	18	21	19	19	24	27	30	26	27	29
	B	10	10	10	10	10	7.6	7.6	7.8	7.4	7.6	7.2	8.00	7.90	8.12	7.92	7.98	8.03	22	18	18	21	19	19	24	28	30	26	27	30
	C	10	10	9	9	9	7.6	7.4	7.8	7.4	7.3	7.2	8.00	7.96	8.13	7.95	8.04	8.07	22	17	18	21	19	19	24	28	30	26	28	30
	D	10	10	10	10	10	7.6	7.5	8.1	7.5	7.3	7.4	8.00	7.85	8.11	7.94	7.83	8.06	22	17	18	21	18	18	24	27	28	25	27	30
	E	10	10	10	10	10	7.6	7.0	8.1	7.4	7.5	7.5	8.00	7.81	8.05	7.93	8.06	8.05	22	17	18	21	18	18	24	27	28	25	26	27
3.1%	A	10	6 (10)	10	10	9	7.4	4.2	7.9	7.4	7.8	4.1	7.95	7.58	8.07	7.84	8.04	7.56	22	18	18	21	19	19	24	25	27	25	26	27
	B	10	7 (10)	10	10	10	7.4	5.9	7.8	7.3	7.8	6.9	7.95	7.71	8.09	7.86	8.02	8.03	22	17	18	21	18	18	24	25	27	25	26	28
	C	10	10	10	10	9	7.4	7.3	4.3	7.3	7.7	7.1	7.95	7.95	7.49	7.73	8.07	8.07	22	18	18	21	18	18	24	25	27	25	26	28
	D	10	10	10	10	9	7.4	4.2	7.7	7.1	7.8	7.1	7.95	7.60	8.04	7.83	8.07	7.87	22	18	18	21	18	18	24	25	26	25	26	26
	E	10	7 (10)	10	10	10	7.4	7.3	7.8	7.2	7.9	7.1	7.95	7.94	8.08	7.84	8.06	8.02	22	18	18	21	18	18	24	25	26	26	26	26
6.25%	A	10	10	10	10	9	7.4	7.4	7.9	7.1	7.9	7.7	7.86	7.97	8.06	7.78	8.03	8.03	22	18	18	21	18	18	24	25	26	25	26	27
	B	10	8 (7)	7	8 (7)	7	7.4	2.5	7.9	7.1	7.8	7.5	7.86	7.99	8.03	7.77	8.08	8.05	22	18	18	21	18	18	24	25	26	25	26	27
	C	10	8 (8)	8	8 (8)	8	7.4	6.5	8.0	7.1	7.8	7.5	7.80	7.90	8.02	7.77	8.08	8.07	22	18	18	21	18	18	24	25	26	25	26	27
	D	10	6 (9)	9	9	8	7.4	7.0	4.9	7.1	7.8	7.5	7.80	7.64	7.18	7.71	8.08	8.05	22	18	18	21	18	18	24	25	26	25	26	27
	E	10	6 (9)	9	9	8	7.4	7.1	4.7	7.0	7.7	7.5	7.80	7.75	7.63	7.72	8.12	8.04	22	18	18	21	18	18	24	25	26	25	26	27
DATE		3/22	3/23	3/24	3/25	3/26	3/22	3/23	3/24	3/24	3/25	3/26	3/22	3/23	3/24	3/24	3/25	3/26	3/22	3/23	3/24	3/24	3/25	3/26	3/22	3/23	3/24	3/24	3/25	3/26
TIME		1555	1545	1600	1540	1600	1505	1545	1530	1620	1530	1530	1505	1545	1530	1620	1530	1530	1505	1545	1530	1620	1530	1530	1505	1545	1530	1620	1530	1530
INITIALS		LH	BC	LH	BC	BC	LH	BC	LH	LH	BC	BC	LH	BC	LH	LH	BC	BC	LH	BC	LH	LH	BC	BC	LH	BC	LH	LH	BC	BC
FED?		✓	✓	✓																		E✱								

✖ - Pull on 50% effluent also.
◇ - "Old" water qualities (prior to renewal)

✦ - AERATE FROM START!
☆ - "New"water qualities (post renewal)

ACUTE BIOASSAY DATA SUMMARY

STUDY: 10213	SAMPLE RECEIVED: 3/22/02	"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES	
CLIENT: CH2M Hill	TEST ORGANISM: *A. bahia*		
SAMPLE: American Samoa	ORGANISM SUPPLIER: ESI	EFFLUENT	
DILUENT: LAB SALT	ORGANISM BATCH/AGE: 1-5days	DILUENT	

	TRC	AMM 0 HR✖	AMM 48 HR✖	pH	DO	Salinity
	See page 1 for details and salinity adjustment record.					

CONC	REP	SURVIVAL 0	24	48	72	96	✦DISSOLVED OXYGEN (MG/L)✦ 0	24	48◊	48☆	72	96	PH (SU) 0	24	48◊	48☆	72	96	TEMPERATURE (°C) 0	24	48◊	48☆	72	96	SALINITY (ppt) 0	24	48◊	48☆	72	96
12.5%	A	10	8	10	10	10	7.1	6.6	4.3	6.2	7.6	7.5	7.68	8.05	7.61	7.58	8.05	8.07	22	18	18	21	18	18	24	25	26	25	26	27
	B	10	8	10	10	10	7.1	6.8	6.0	6.2	7.6	7.4	7.68	7.99	7.88	7.59	8.13	8.14	22	18	18	21	18	18	24	25	26	25	26	27
	C	10	0	–	–	–	7.1	6.9	–	–	–	–	7.68	7.82	–	–	–	–	22	18	–	–	–	–	24	25	–	–	–	–
	D	10	9	9	9	5	7.1	6.1	6.1	6.4	7.3	7.5	7.68	7.81	7.61	7.59	8.07	8.11	22	18	18	21	18	18	24	25	26	25	26	27
	E	10	9	9	9	9	7.1	6.5	7.6	6.3	7.4	3.2	7.68	7.82	8.11	7.67	7.74	7.68	22	18	18	21	18	18	24	25	26	25	26	27
25%	A	10	8	10	10	8	6.9	6.9	4.6	6.6	7.4	6.6	7.44	8.0	7.72	7.42	8.08	8.02	21	18	18	21	18	18	24	25	26	25	26	27
	B	10	2	8	8	5	6.9	7.0	3.8	5.7	7.3	7.0	7.44	7.75	7.65	7.40	8.06	8.16	21	18	18	21	18	18	24	25	27	25	26	27
	C	10	0	–	–	–	6.9	7.1	–	–	–	–	7.44	7.41	–	–	–	–	21	18	–	–	–	–	24	26	–	–	–	–
	D	10	0	–	–	–	6.9	0.5	–	–	–	–	7.44	7.46	–	–	–	–	21	18	–	–	–	–	24	25	–	–	–	–
	E	10	0	–	–	–	6.9	0.9	–	–	–	–	7.44	7.69	–	–	–	–	21	19	–	–	–	–	24	25	–	–	–	–
50%	A	10	0	–	–	–	5.8	0.5	–	–	–	–	7.16	7.87	–	–	–	–	21	19	–	–	–	–	24	26	–	–	–	–
	B	10	0	–	–	–	5.8	0.8	–	–	–	–	7.16	7.91	–	–	–	–	21	18	–	–	–	–	24	26	–	–	–	–
	C	10	0	–	–	–	5.8	0.5	–	–	–	–	7.16	7.98	–	–	–	–	21	18	–	–	–	–	24	26	–	–	–	–
	D	10	0	–	–	–	5.8	0.1	–	–	–	–	7.16	7.52	–	–	–	–	21	19	–	–	–	–	24	26	–	–	–	–
	E	10	0	–	–	–	5.8	0.5	–	–	–	–	7.16	7.97	–	–	–	–	21	19	–	–	–	–	24	26	–	–	–	–
DATE		3/23	3/23	3/24	3/25	3/26	3/23	3/23	3/24	3/24	3/25	3/26	3/23	3/23	3/24	3/24	3/25	3/26	3/23	3/23	3/24	3/24	3/25	3/26	3/23	3/23	3/24	3/24	3/25	3/26
TIME		1555	1545	1600	1540	1600	1505	1545	1530	1620	1530	1530	1505	1545	1530	1620	1530	1530	1505	1545	1530	1620	1530	1530	1505	1545	1530	1630	1550	1530
INITIALS		WS	BC	WS	BV	BC	WS	BC	WS	WS	BV	BC	WS	BC	WS	WS	BV	BC	WS	BC	WS	WS	BV	BC	WS	BC	WS	WS	BV	BC
FED?		✓	✓	✓																										

✖ - Pull on 50% effluent also.

◊ - "Old" water qualities (prior to renewal)

✦ - AERATE FROM START!

☆ - "New"water qualities (post renewal)

****

STUDY NO.: 10213 ASSAY START: 03/22/02

PECIES: A. bahia EXPOSURE: 24 hours

SAMPLE: CH2M Hill Am Samoa

****

The binomial test shows that 12.5 and 50 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits s greater than 95 percent.

An approximate LC50 of 19.72354 is obtained by nonlinear interpolation between 12.5 and 25

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

Span	G	LC50	95 Percent Confidence Limits	
4	1.860789E-02	15.87357	13.99062	18.17385
3	3.259931E-02	17.15707	14.86537	19.76222
2	3.938606E-02	19.35455	16.77484	21.86717
1	.2300398	19.72353	16.66293	24.92509

An LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
4	.4333274	3.906349	11.71905	8.409798E-03

s the probability associated with this value is <0.05, results should be used with caution.

Slope = 3.310178
95 Percent Confidence Limits = 1.131167 and 5.489188

LC50 = 16.88444
95 Percent Confidence Limits = 9.355951 and 32.45659

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD------

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
25	3.218876	64	.64	.675
12.5	2.525729	24	.24	.175
6.25	1.832581	16	.16	.075
3.1	1.131402	0	0	-.125 *

Alpha = 10 %
Groups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.
LC50 = 21.7138
Estimated 95 Percent Confidence Limits
Lower: 18.31288 Upper: 25.74631
Variance estimate = 7.254201E-03

****

STUDY NO.: 10213 ASSAY START: 03/22/02

SPECIES: A. bahia EXPOSURE: 24 hours

SAMPLE: CH2M Hill Am Samoa

****

SUMMARY TABLE

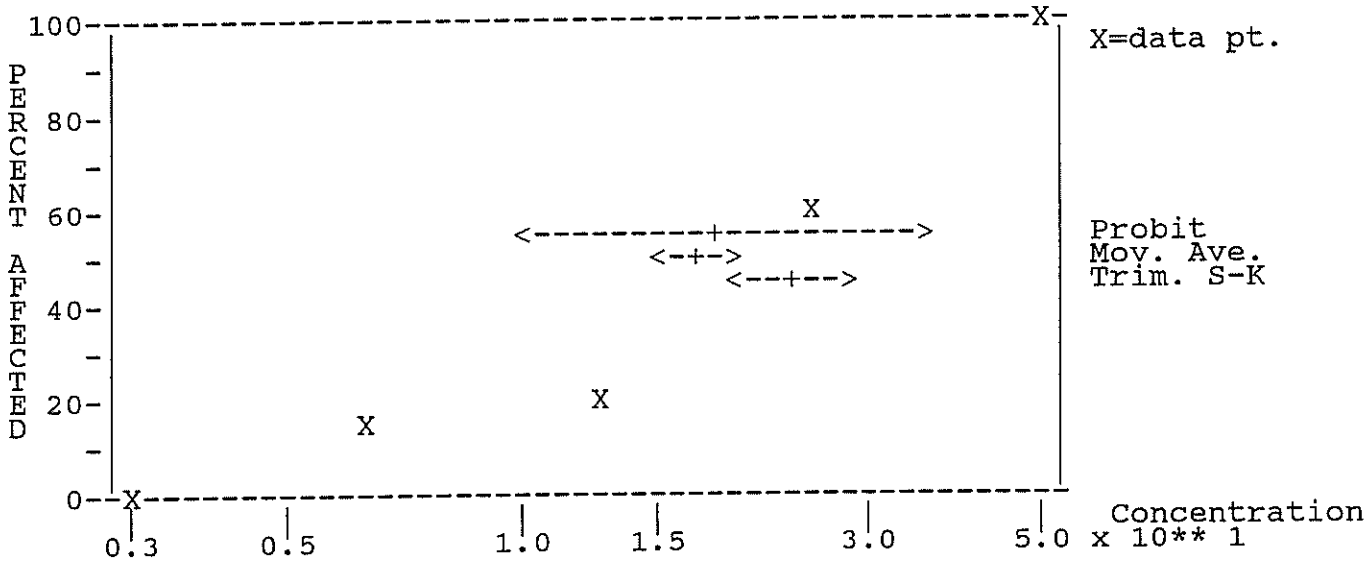
04-28-2002 09:25:41

Data:	Conc.	Exposed	Dead	Percent
	50	50	50	100
	25	50	32	64
	12.5	50	12	24
	6.25	50	8	16
	3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	16.88444	9.355951	32.45659	
Moving Average	15.87357	13.99062	18.17385	Span= 4
Spearman-Karber	21.7138	18.31288	25.74631	Alpha= 1(
Binomial	19.72354			

Compare results with original data to see if they are reasonable.

****



****

TUDY NO.: 10213 ASSAY START: 03/22/02

SPECIES: A. bahia EXPOSURE: 48 hours

SAMPLE: CH2M Hill

****

The binomial test shows that 12.5 and 50 can be used
s statistically sound, conservative 95 percent confidence limits
ecause the actual confidence level associated with these limits
s greater than 95 percent.

An approximate LC50 of 19.72354 is obtained by
onlinear interpolation between 12.5 and 25

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

Span	G	LC50	95 Percent Confidence Limits	
4	1.860789E-02	15.87357	13.99062	18.17385
3	3.259931E-02	17.15707	14.86537	19.76222
2	3.938606E-02	19.35455	16.77484	21.86717
1	.2300398	19.72353	16.66293	24.92509

An LC50 calculated using the Moving Average method may not be a very goo
stimate if the span is much less than the number of concentrations.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
4	.4333274	3.906349	11.71905	8.409798E-03

As the probability associated with this value is <0.05, results should
be used with caution.

Slope = 3.310178
95 Percent Confidence Limits = 1.131167 and 5.489188

LC50 = 16.88444
95 Percent Confidence Limits = 9.355951 and 32.45659

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD----

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
25	3.218876	64	.64	.675
12.5	2.525729	24	.24	.175
6.25	1.832581	16	.16	.075
3.1	1.131402	0	0	-.125 *

Alpha = 10 %
Groups trimmed and therefore not used in estimating LC50
are marked with an asterisk above.
LC50 = 21.7138
Estimated 95 Percent Confidence Limits
Lower: 18.31288 Upper: 25.74631
Variance estimate = 7.254201E-03

****

STUDY NO.: 10213 ASSAY START: 03/22/02

SPECIES: A. bahia EXPOSURE: 48 hours

SAMPLE: CH2M Hill

****

SUMMARY TABLE

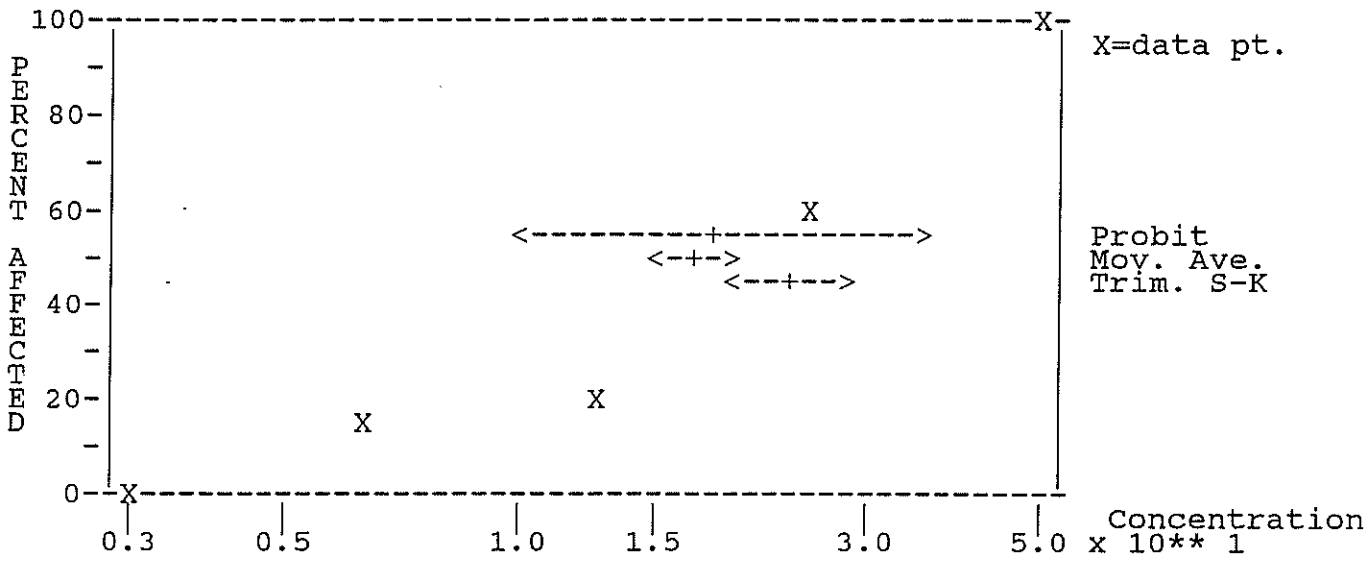
04-28-2002 09:37:04

Data:	Conc.	Exposed	Dead	Percent
	50	50	50	100
	25	50	32	64
	12.5	50	12	24
	6.25	50	8	16
	3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	16.88444	9.355951	32.45659	
Moving Average	15.87357	13.99062	18.17385	Span= 4
Spearman-Karber	21.7138	18.31288	25.74631	Alpha= 10
Binomial	19.72354			

Compare results with original data to see if they are reasonable.

****



****

TUDY NO.: 10213 ASSAY START: 03/22/02

PECIES: A. bahia EXPOSURE: 72 hours

AMPLE: CH2M Hill American Samoa

****

The binomial test shows that 12.5 and 50 can be used
s statistically sound, conservative 95 percent confidence limits
ecause the actual confidence level associated with these limits
s greater than 95 percent.

An approximate LC50 of 19.72354 is obtained by
onlinear interpolation between 12.5 and 25

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

Span	G	LC50	95 Percent Confidence Limits	
4	1.860789E-02	15.87357	13.99062	18.17385
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An LC50 calculated using the Moving Average method may not be a very good
stimate if the span is much less than the number of concentrations.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
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As the probability associated with this value is <0.05, results should
be used with caution.

lope = 3.310178
95 Percent Confidence Limits = 1.131167 and 5.489188

C50 = 16.88444
95 Percent Confidence Limits = 9.355951 and 32.45659

---------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-----

onc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
25	3.218876	64	.64	.675
12.5	2.525729	24	.24	.175
6.25	1.832581	16	.16	.075
3.1	1.131402	0	0	-.125 *

Alpha = 10 %
Groups trimmed and therefore not used in estimating LC50
re marked with an asterisk above.
LC50 = 21.7138
Estimated 95 Percent Confidence Limits
Lower: 18.31288 Upper: 25.74631
Variance estimate = 7.254201E-03

****

STUDY NO.: 10213 ASSAY START: 03/22/02

SPECIES: A. bahia EXPOSURE: 72 hours

SAMPLE: CH2M Hill American Samoa

****

SUMMARY TABLE

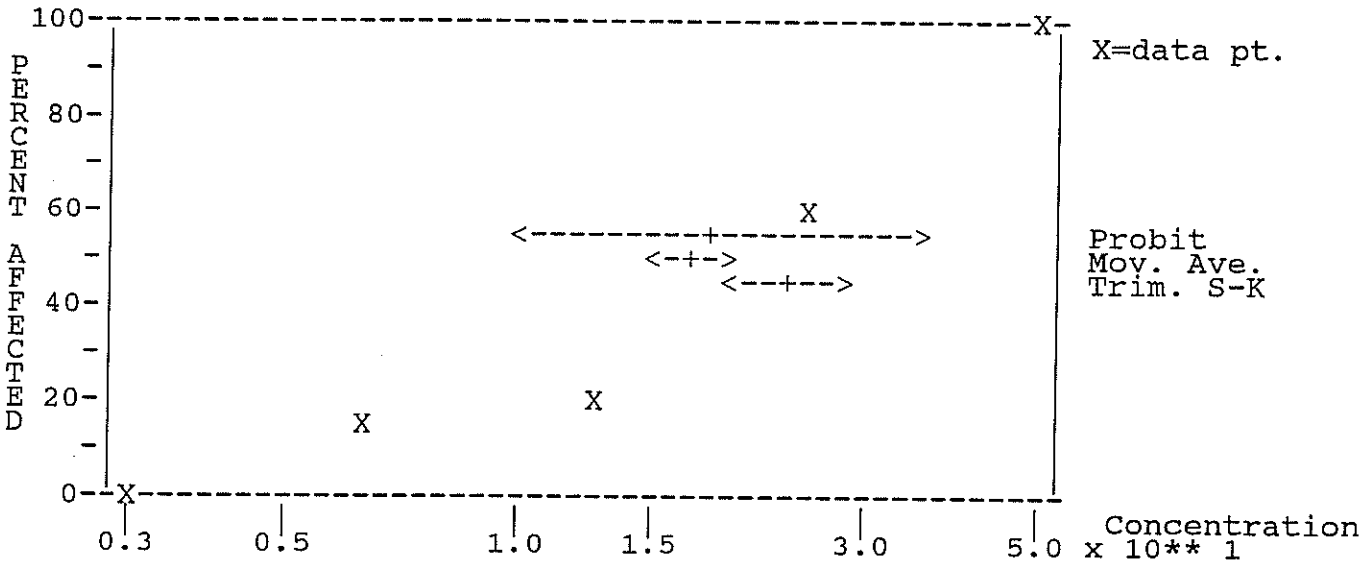
04-28-2002 09:41:01

Data: Conc.	Exposed	Dead	Percent
50	50	50	100
25	50	32	64
12.5	50	12	24
6.25	50	8	16
3.1	50	0	0

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	16.88444	9.355951	32.45659	
Moving Average	15.87357	13.99062	18.17385	Span=
Spearman-Karber	21.7138	18.31288	25.74631	Alpha=
Binomial	19.72354			

Compare results with original data to see if they are reasonable.

****



STUDY NO.: 10213 ASSAY START: 03/22/02

PECIES: A. bahia EXPOSURE: 96 hours

SAMPLE: CH2M Hill American Samoa

ne binomial test shows that 12.5 and 25 can be used as statistically sound, conservative 95 percent confidence limits because the actual confidence level associated with these limits s greater than 95 percent.

an approximate LC50 of 16.7708 is obtained by nonlinear interpolation between 12.5 and 25

----------RESULTS CALCULATED USING THE MOVING AVERAGE METHOD----------

pan	G	LC50	95 Percent Confidence Limits	
4	2.491899E-02	13.61022	11.76908	15.808
3	3.587326E-02	14.78996	12.60099	17.1303
2	.1214662	16.31729	13.22481	21.67258
1	.2101414	16.7708	13.79452	19.82581

an LC50 calculated using the Moving Average method may not be a very good estimate if the span is much less than the number of concentrations.

----------RESULTS CALCULATED USING THE PROBIT METHOD----------

Iterations	G	H	Chi-Square	Probability
4	.2967415	2.771085	8.313255	3.996211E-02

s the probability associated with this value is <0.05, results should e used with caution.

Slope = 2.841277
95 Percent Confidence Limits = 1.29352 and 4.389035

LC50 = 13.86267
95 Percent Confidence Limits = 8.400122 and 23.35912

--------RESULTS CALCULATED USING THE TRIMMED SPEARMAN-KARBER METHOD-------

Conc.	Dose =Ln (Conc)	Percent Dead	Monotonic Rel. Freq.	Trimmed Rel. Freq.
50	3.912023	100	1	1.125 *
25	3.218876	74	.74	.8
12.5	2.525729	32	.32	.275
6.25	1.832581	20	.2	.125
3.1	1.131402	6	.06	-5.000001E-02 *

Alpha = 10 %
roups trimmed and therefore not used in estimating LC50 are marked with an asterisk above.
LC50 = 16.12887
Estimated 95 Percent Confidence Limits
Lower: 13.48165 Upper: 19.29588
Variance estimate = 8.035425E-03

STUDY NO.: 10213 ASSAY START: 03/22/02

SPECIES: A. bahia EXPOSURE: 96 hours

SAMPLE: CH2M Hill American Samoa

****

SUMMARY TABLE

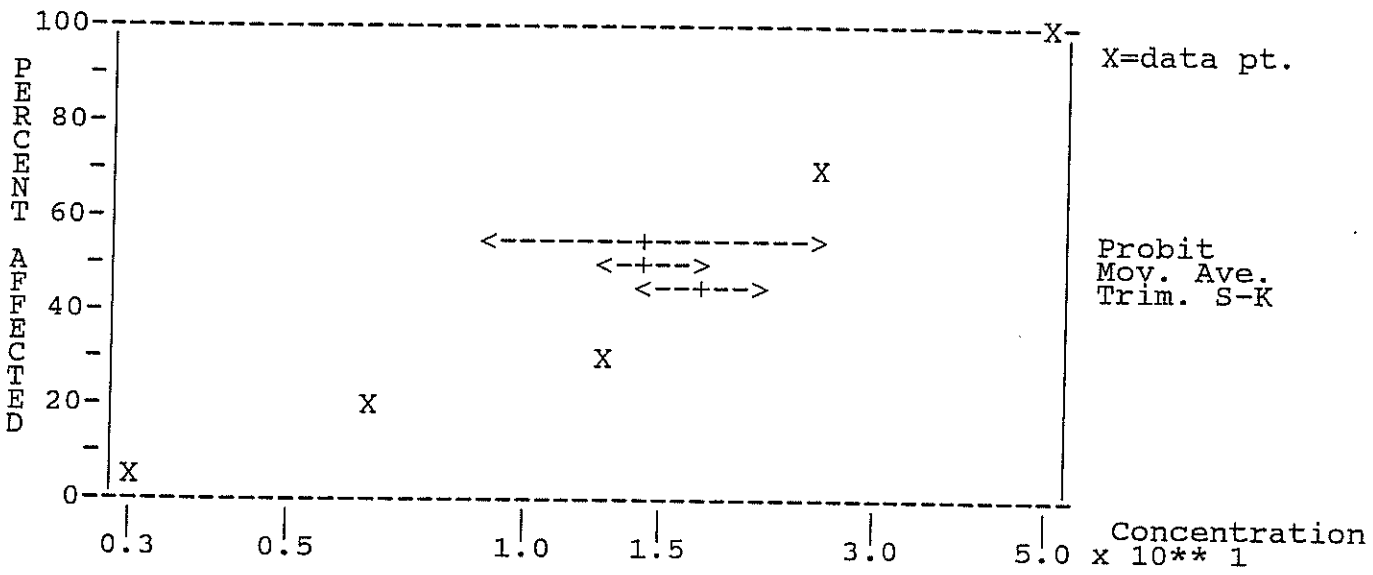
04-22-2002 09:57:43

Data: Conc.	Exposed	Dead	Percent
50	50	50	100
25	50	37	74
12.5	50	16	32
6.25	50	10	20
3.1	50	3	6

	LC50	Lower 95% Limit	Upper 95% Limit	
Probit Analysis	13.86267	8.400122	23.35912	
Moving Average	13.61022	11.76908	15.808	Span= 4
Spearman-Karber	16.12887	13.48165	19.29588	Alpha= 10
Binomial	16.7708			

Compare results with original data to see if they are reasonable.

****



EnviroSystems, Incorporated
Organism Culture and Acclimation Data

I. Organism Information

Species: A. bahia Age: 1-5 days

Client: Chamth'l ESI#: 10213

Culturist: ESI Date: 3/22/02

II. Culture Maintenance Information

Flow Through _____ Glass Static _____

Brood Board _____ Static Renewal _____

Hatch Date: _____ Lot #: _____

Brood Organism: _____

III. Acclimation History

Temperature: _____ °C Salinity: _____ ppt

Hardness: _____ mg/L as CaCO3 pH: _____ SU

DO: _____ mg/L

Water Type: _____

SITE WATER ACCLIMATION (If applicable)

Start: _____ End: _____

RECORD OF METERS USED FOR WATER QUALITY MEASUREMENTS

STUDY: 10213	CLIENT: CH2M HILL - American Samoa					
WATER QUALITIES - A. bahia						
HOURS:	0	24	48 - old	48 - new	72	96
Water Quality Station #	1	1	1	1	2	
Initials	HH	BC	HH	HH	BV	
Date	3/22/07	3/23/07	3/24/07	3/24/07	3/25/07	

Water Quality Station #1		Water Quality Station #2		COMMENTS
DO meter #	21	DO meter #	19	
DO probe #	3	DO probe #	1	
pH meter #	1138	pH meter #	50	
pH probe #	27	pH probe #	28	
S/C meter #	45130	S/C meter #	45130	
S/C probe #	1	S/C probe #	1	
Salinity meter #	45130	Salinity meter #	45130	

PREPARATION OF DILUTIONS

STUDY: 10213	CLIENT: CH2M HILL - American Samoa
SPECIES: *A. bahia*	

Diluent: Lab Salt	Day: 0 Sample: EOA		Day: 2 Sample: EOA					
Concentration	Vol. Eff.	Final Vol	Vol. Eff.	Final Vol	HRS	Date	Time	Initials
LAB	0	1000mL	0	1000	0	3/22/02	1500	MA
3.1%	31		31		48	3/24	1610	MA
6.25%	62.5		62.5					
12.5%	125		125					
25%	250		100	400				
50%	500		—	—				

Comments:

ESI SAMPLE RECEIPT RECORD

EnviroSystems Inc.
P.O. Box 778 One Lafayette Road
Hampton, N.H. 03843-0778
(603) 926-3345 • (603) 926-3521 Fax
E-mail ESI @ www.envirosystems.com

ESI STUDY NUMBER: 10213

SAMPLE RECEIPT DATE: 3/22/02 TIME: 1325

SAMPLE RECEIVED BY: DH

DELIVERED VIA: ☐ FEDEX ☐ CLIENT ☐ ESI ☐ UPS ☒ OTHER

SAMPLE CONDITION:

CHAIN OF CUSTODY:	☑ YES	☐ NO
CHAIN OF CUSTODY SIGNED:	☑ YES	☐ NO
CHAIN OF CUSTODY COMPLETE:	☐ YES	☑ NO
SAMPLE DATE:	☑ YES	☐ NO
SAMPLE TIME RECORDED:	☐ YES	☑ NO
SAMPLE TYPE IDENTIFIED:	☑ YES	☐ NO
CUSTODY SEAL IN PLACE:	☑ YES	☐ NO
SHIPPING CONTAINER INTACT:	☑ YES	☐ NO

SAMPLE TEMPERATURE (AT ARRIVAL): 15 °C

COMMENTS: 1 x 5 gal ESF

COOLER NUMBER: _____

CH2M HILL
APPLIED SCIENCES LABORATORY

CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES

CH2M Hill Project # 147323.JC.02.NT
Purchase Order #
Project Name: JOINT CANNERY OUTFALL SAMOA
Company Name/CH2M HILL Office
Project Manager & Phone #: Mr. [ ] Ms. [ ] Dr. [ ] STEVE COSTA 707-677-0123
Report Copy to: SAME
Requested Completion Date:
Sampling Requirements: SDWA ☐ NPDES ☐ RCRA ☐ OTHER ____
Sample Disposal: Dispose ☐ Return ☐

LAB TEST CODES

SHADED AREA-- FOR LAB USE ONLY
Lab 1 # | Lab 2 #
Quote # | Kit Request #
Project #
No. of Samples | Page of
Login | LIMS Ver

ANALYSES REQUESTED

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	Matrix AIR	CLIENT SAMPLE ID (9 CHARACTERS)	# OF CONTAINERS	MYSID ACUTE (96hr) BIOASSAY	REMARKS	LAB 1 ID	LAB 2 ID
3/14/02		X		X			JC0-02-NT	1	X	Dilutions 5%, 25%, 12½%, 6¼%, 3⅛		

Sampled By & Title: Client (Please sign and print name) — Date/Time: 3/14-16/02
Received By (Please sign and print name) — Date/Time
Received By: K. Hawkins (Please sign and print name) — Date/Time: 3/22/02 1305
Received By (Please sign and print name) — Date/Time
Work Authorized By (Please sign and print name)

Relinquished By: Steve Costa (Please sign and print name) — Date/Time: 3/15/02
Relinquished By (Please sign and print name) — Date/Time
Relinquished By (Please sign and print name) — Date/Time
Shipped Via: UPS BUS Fed-Ex Hand Other DHL — Shipping # 8190660203
Remarks

QC Level: 1 2 3 Other: ____
COC Rec | ICE
Ana Req | TEMP
Cust Seal | Ph

Instructions and Agreement Provisions on Reverse Side

DISTRIBUTION: Original - LAB Yellow - LAB Pink - Client

State of New Hampshire
Environmental Laboratory Accreditation Program

Awards Primary Accreditation to

EnviroSystems, Inc.
of
Hampton, NH

For the analyses listed on the attached page(s) in accordance with the provisions of the NELAC Standards and Env-C 300.

Certificate Number: 151301

Date of Issue: December 21, 2001

Expiration Date: December 20, 2002

Program Manager

Continuing accreditation status is dependent on successful ongoing participation in the program.
Customers may verify the laboratory's current status by calling (603) 271-2991 or (603) 271-2998

Laboratory: Envirosystems, Inc. Address: One Lafayette Road, Hampton, NH 03843 (603) 926-3345
Certificate Number: 151301-A Date of Issue: 12/21/01 Expiration Date: 12/20/02 Page 1 of 1

ACCREDITS THE ABOVE MENTIONED LABORATORY FOR THE FOLLOWING ANALYSES:

WASTEWATER MICROBIOLOGY

Fecal Coliform	SM 9222 D

WASTEWATER METALS

Aluminum:	EPA 200.7
Arsenic:	EPA 200.7
Cadmium:	EPA 200.7
Chromium:	EPA 200.7
Copper:	EPA 200.7
Iron:	EPA 200.7
Lead:	EPA 200.7
Manganese:	EPA 200.7
Nickel:	EPA 200.7
Selenium:	EPA 200.7
Silver:	EPA 200.7
Vanadium:	EPA 200.7
Zinc:	EPA 200.7

WASTEWATER INORGANIC CONTAMINANTS

Alkalinity:	EPA 310.1
Ammonia-N:	SM 4500-NH_3 F
Calcium:	EPA 200.7
Hardness:	EPA 200.7
Magnesium:	EPA 200.7
pH:	SM 4500 H^+ B
Residual Chlorine, Total:	SM 4500 Cl D
Residue, Total:	SM 2540 B
Conductivity	SM 2510 B
TOC	SM 5310 C

FRESH WATER ACUTE TOXICITY TESTING

Ceriodaphnia dubia:	EPA/600/4-90/027F
Daphnia pulex:	EPA/600/4-90/027F
Daphnia magna:	EPA/600/4-90/027F
Pimephales promelas:	EPA/600/4-90/027F
Oncorhynchus mykiss:	EPA/600/4-90/027F
Salvelinus fontinalis:	EPA/600/4-90/027F
Cyprinella leedsi:	EPA/600/4-90/027F

SALT WATER ACUTE TOXICITY TESTING

Mysidopsis bahia:	EPA/600/4-90/027F
Cyprinodon variegatus:	EPA/600/4-90/027F

SALT WATER ACUTE TOXICITY TESTING (Cont.)

Menidia beryllina:	EPA/600/4-90/027F
Menidia Menidia:	EPA/600/4-90/027F
Menidia peninsulae:	EPA/600/4-90/027F
Holmesimysis costata:	EPA/600/4-90/027F

FRESH WATER CHRONIC TOXICITY TESTING

Pimephales promelas:	EPA 1001
Ceriodaphnia dubia:	EPA 1002
Selenastrum capricornutum:	EPA 1003

SALT WATER CHRONIC TOXICITY TESTING

Cyprinodon variegatus:	EPA 1004
Cyprinodon variegatus:	EPA 1005
Menidia beryllina:	EPA 1006
Mysidopsis bahia:	EPA 1007
Arbacia punctulata:	EPA 1008
Champia parvula:	EPA 1009

This certificate supercedes all previously issued certificates.

Program Manager